Testing Their Metal

Metals continually rank at the top of the U.S. Environmental Protection Agency's annual list of agents that pose the greatest hazard to the people of the United States. Metals aren't going away, either. They do not biodegrade, and they often concentrate in human and animal cells and tissue. Many metals are known human and animal carcinogens, while many others are suspected to play a role in cancer. Little is known, however, about the mechanisms by which metals cause cancer.

The Inorganic Carcinogenesis Section (ICS) at the NIEHS, headed by toxicologist Michael Waalkes, is devoted to studying the carcinogenic mechanisms of metals. The section is a collaborative effort between the Laboratory of Pharmacology and Chemistry at the NIEHS and the Laboratory of Comparative Carcinogenesis at the National Cancer Institute (NCI). The section was previously located at the NCI and was moved to the NIEHS in 1996. Waalkes, who served as chief of the section at the NCI, says he was sent from that agency to act as a bridge and stimulate collaboration between the two institutes. Says Waalkes, "We study how tolerance develops to these metals. Sometimes they are stored, occasionally they are metabolized. It's a totally different world than [studying] organics."

The ICS is specifically focusing on studying three inorganics: arsenic, cadmium, and lead. Arsenic and cadmium are classified as known human carcinogens by the International Agency for Research on Cancer and the National Toxicology Program, and lead is considered a possible human carcinogen. Ironically, despite their carcinogenic activity, arsenic and cadmium also have antitumor potential, and arsenic is already used in chemotherapy for specific tumors. The ICS is also studying this capacity.

"All of us are exposed to these metals; it's just a fact of life," says Waalkes. "It's important for us to understand the mechanisms by which they are carcinogenic, in order to assess the risks and take preventive measures if necessary."

Flame readers. Toxicologist Michael Waalkes and lab technician Tammy Knowles examine the flame from an atomic absorption spectrophotometer, which is used to measure the levels of metals in biological samples.

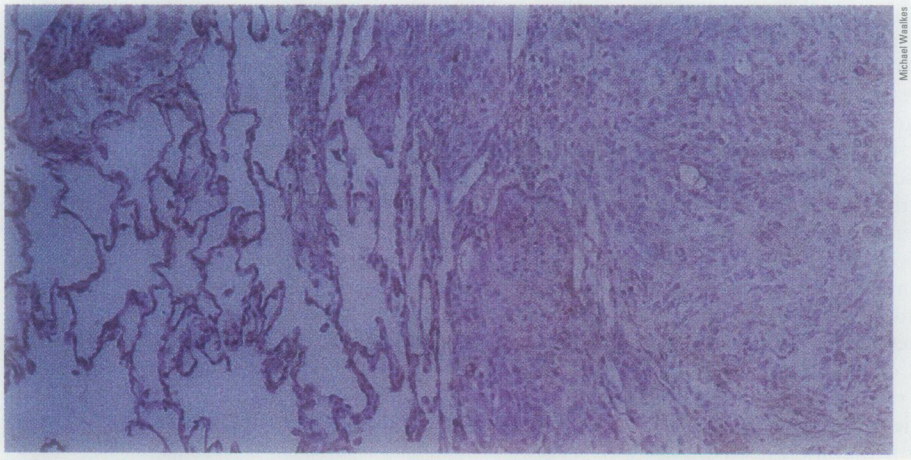
Arsenic. For more than a century, scientists have known that arsenic is a human carcinogen, but its mechanism of action is still unknown. It has been associated with lung, skin, bladder, and liver tumors. Arsenic is a by-product of many industrial processes, including semiconductor manufacturing, petroleum refining, and mining and smelting operations. Arsenic is commonly found in nature and is released from soil and rock erosion into water. High levels of arsenic are prevalent in drinking water in many areas, including the southwestern United States, Michigan, and southern Asia. Currently, there is controversy about what level of arsenic in drinking water is safe, as the health effects of low levels of arsenic exposure are unclear.

Waalkes says arsenic's mechanism of carcinogenic action is difficult to study. It is the only known human carcinogen that has not been definitely shown to cause tumors by itself in laboratory animals. This indicates that humans may be more sensitive to arsenic than animals.

Waalkes and colleagues had a breakthrough finding when they used a cell model system to test a hypothesis about the potential role of methylation in arsenic's mechanism of carcinogenic action. When arsenic is metabolized, it undergoes enzymatic methylation, a process in which methyl groups are added to the metal. This process is thought to aid in detoxifying the metal.

DNA is also typically methylated in order to serve in regulating the expression of a variety of genes, including oncogenes. However, DNA methylation requires the same methyl donor that is consumed in arsenic methylation. When the methyl donor is not available, the DNA is undermethylated, or hypomethylated, and is therefore unable to properly regulate gene expression. Therefore, arsenic appears to indirectly induce aberrant gene expression through the induction of DNA hypomethylation. DNA hypomethylation is thought to constitute an early event in some cancers and has been associated with many types of tumors, including those of the liver and colon.

Waalkes and colleagues tested this hypothesis and successfully confirmed that malignant transformation occurred in cultured epithelial liver cells of rats. Malignant transformation was confirmed by the production of tumors when the cells were injected into mice. This study was published in the 30 September 1997 issue of the *Proceedings of the National Academy of Sciences*. The researchers are currently investigating gene activation during arsenic-induced malignant transformation in this cultured cell system. They are also studying the carcinogenic effects of arsenic in mice in several specialized systems, including transplacental exposure.



Michael Waalkes

In the case of cadmium. A micrograph of the lung of a rat injected with cadmium shows a metastatic fibrosarcoma on the right. On the left are normal cells.

Cadmium. Although cadmium is carcinogenic, it is not thought to be highly

mutagenic. How cadmium causes cancer is still unknown, but Waalkes and colleagues are making significant strides in unraveling the mystery of this mechanism. Cadmium is used in electroplating, paints, and cadmium–nickel batteries.

In a significant 1998 study, ICS researchers found that cadmium appears to block programmed cell death, or apoptosis. By stopping this process, cells that would otherwise die, including those with damaged DNA, may continue to live and divide. This probably results in increasing numbers of cells with altered DNA, which can eventually lead to the development of tumors. The researchers believe that the suppression of apoptosis may be a significant aspect of cadmium's carcinogenic mechanism. The study was published in the 22 May 1998 issue of the *Journal of Toxicology and Environmental Health.*

Some research suggests that cadmium exposure may be associated with human prostate cancer. Both the incidence and mortality of prostate cancer is increasing in the United States. Age-adjusted mortality rates from prostate cancer have increased by nearly 30% over the last 35 years. The exact cause of prostate cancer is still unknown, but demographic studies suggest that environmental factors, perhaps including exposure to cadmium, may play a role.

Waalkes and colleagues are working to develop models of cadmium-induced prostate cancers. In several studies, they discovered that cadmium exposure was associated with proliferative lesions and cancers of the rat prostate. One recent study has been submitted to *Toxicological Sciences* for publication.

Waalkes says his team will continue to look at model systems of the human prostate, and they plan to test a hypothesis that cadmium may modify proliferation in the prostate epithelial cells by altering the way that testosterone interacts with its receptor.

Lead. Lead is considered one of the single most hazardous substances to the population of the United States due to its neurotoxicity, particularly in children, says Waalkes. However, its carcinogenic potential remains controversial. Lead has accumulated in houses and the environment due to its use for many years in paint and gasoline.

In animals, lead was previously thought to be carcinogenic in the kidney as a result of nongenotoxic mechanisms involving chronic nephropathy. This process occurs when DNA, in response to a toxic stimulus, attempts to repair damage caused by the toxicant. The repair attempts cause increases in the rate of cell division, which in turn increases the incidence of random errors in DNA. These errors have the potential to lead to the development of tumors.

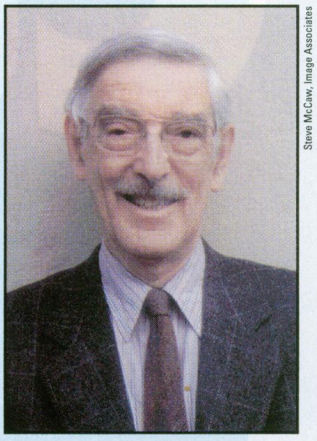
However, ICS researchers examined the carcinogenicity of short-term gestational and lactational lead exposure in mice and found that lead was clearly a renal carcinogen in treated offspring. In what Waalkes calls "a major breakthrough," the team found that lead-induced renal tumors occurred long after short-term exposure and in the absence of chronic neuropathy. The researchers say this indicates that lead may be a specific renal carcinogen. These findings were published in the 15 November 1995 issue of *Cancer Research.* Waalkes adds that lead was one of the first inorganic substances shown to be a transplacental carcinogen in rodents. Future studies will help elucidate the potential genotoxic mechanisms of lead, Waalkes says.

Future plans for the ICS also include the study of metallothionein, a metal-binding protein that is involved in protection against metal toxicity. ICS researchers have conducted studies on the role of metallothionein in the antitumor effects of cadmium. They plan to further investigate metallothionein's role in arsenic-induced malignant transformation and in lead-induced renal tumors.

Brandy E. Fisher

Ames Awarded Medal of Honor

Bruce Ames, a professor of biochemistry and molecular biology and director of the NIEHS Center at the University of California at Berkeley, was awarded the nation's top scientific honor—the National Medal of Science. Ames was given the award by President Clinton on 27 April 1999.



Steve McCaw, Image Associates

The National Medal of Science was established in 1959 and is given annually to individuals for outstanding contributions to the knowledge base of the physical, biological, mathematical, engineering, social, and behavioral sciences. The National Science Foundation administers the program for the President.

Ames was chosen for work that "changed the direction of basic and applied research on mutation, cancer, and aging," according to a press release from the White House Office of Science and Technology Policy. Ames says, "I am interested in what damages DNA and how to prevent it." He says his work indicates that normal metabolism, bad diet, and smoking are the main causes of cancer and other diseases associated with aging.

Ames has conducted extensive research on how micronutrient deficiencies affect health. Research indicates that the quarter of the population that eats the fewest fruits and vegetables has about double the cancer rates for most types of cancer when compared to the quarter with the highest intake. Approximately 80% of U.S. children and adolescents and 68% of adults do not eat the recommended five portions of fruits and vegetables per day. Ames has focused his research on the mechanisms by which micronutrient deficiencies cause DNA damage, which appear to be similar to the cancer-causing mechanisms of radiation and many chemicals.

Ames investigated folate deficiency, which occurs in approximately 10% of the population and in a much higher percentage among the poor. He discovered that folate deficiency causes extensive incorporation of uracil into human DNA, which leads to breaks in chromosomes. This mechanism is the likely cause of the increased cancer risk associated with folate deficiency, Ames says, and may contribute to cognitive defects, which have been associated with low folate intake. Ames says his current research suggests that deficiencies of vitamins B-12 and B-6 also cause high uracil and chromosome breaks.

Ames has also conducted extensive research on oxidants and antioxidants. The oxidant by-products of normal energy metabolism (superoxide, hydrogen peroxide, and hydroxyl radical) are the same mutagens produced by radiation. Deficiencies of dietary antioxidants, such as vitamins C and E, mimic radiation exposure. Ames found that oxidative damage to DNA and other macromolecules appears to play a major role in aging and degenerative diseases associated with aging, including cancer.

Ames was also recognized for his early contribution to the field of genetic toxicology. In the late 1960s, he developed a simple, inexpensive bacterial test, known as the Ames test, for environmental and natural mutagens.

Says Ames, "It's nice when people appreciate you and your work." He plans to persist at unraveling the mysteries of DNA damage, saying, "I'm still busily working in the lab."